Pergamon

0098-8472(93)E0002-8

Environmental and Experimental Botany, Vol. 34, No. 2, pp. 201–206, 1994
Elsevier Science Ltd
Printed in Great Britain
0098-8472/94 $6.00 + 0.00

METABOLIC ACTIVITY OF COTTON ROOTS IN RESPONSE TO TEMPERATURE

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(*Received* 4 *May* 1993; *accepted in revised form* 5 *October* 1993)

McMichael B. L. and Burke J. J. *Metabolic activity of cotton roots in response to temperature.* Environmental and Experimental Botany **34,** 201–206, 1994.—Root growth, under genetic control, responds to numerous environmental stimuli. The occurrence of below optimal soil temperatures for root growth of cotton (*Gossypium hirsutum* L.) at planting time may delay seedling establishment and reduce seasonal crop productivity. The present study assessed cotton root growth and metabolism at various temperatures to determine if observed temperature responses were related to developmental changes in seedling growth. Studies monitoring seedling root growth revealed distinct temperature optima for the cotton seedling. Analysis of the temperature characteristics of *in vivo* mitochondrial electron transport measured by 2,3,5-triphenyltetrazolium chloride reduction showed that the temperature optima of root metabolism at 10 days after planting (DAP) was lower than that obtained from the measure of accumulated root growth at 10 DAP. The differences in the temperature optima appear to be associated with dynamic changes in seedling development which may be related to changes in stored seed reserves. Metabolic temperature responses are broad during peak seed reserve mobilization and become narrow with the depletion of available reserves. Measurement of root length or root number at 10 DAP would reflect a composite of narrow and broad metabolic temperature sensitivities. Because root development is linked to this composite of metabolic temperature responses, the temperatures providing the maximum root size at 10 DAP are actually higher than the optimum temperature for metabolism under the non-saturating substrate levels associated with the majority of the growing season. Evaluation of cotton root growth responses to shoot and root temperatures within or below cotton's thermal kinetic window revealed enhanced root growth when the roots temperatures were within the thermal kinetic window. These findings provide new insights for evaluation of the temperature characteristics of root growth.

INTRODUCTION

The soil temperatures at planting in the Southern Great Plains of the United States may be low enough to hinder the establishment of cotton (*Gossypium hirsutum* L.) seedlings and may reduce plant productivity.[5,6,8,13] The optimum temperatures for the growth of cotton hypocotyls and root systems have been shown to be 36 and 30–33°C, respectivly.[1,10] However, soil temperatures of 12–15°C that may persist for as long as 10–15 days in early May, are common.[6] Wanjura *et al.*[14] showed that when the minimum soil temperature at planting depth (3–5 cm) dropped from approximately 20 to 12°C, the hours required for initial seedling emergence increased from 100 to approximately 425 hours. Research on the genetic variability of the temperature response of cotton root systems in relation to potential plant establishment is lacking. In other species, Williams[15] showed a genotypic response to different root temperature in clover and attributed at least a portion of the changes in response to changes in leaf area. TACHIBANA[13] showed that one cultivar of cucumber was more sensitive to low temperature (12°C) than another and that figleaf gourd was more tolerant to low temperature (12°C) than cucumber.

Neither the biochemical basis for the response of cotton to low temperature nor the interaction of potential metabolic activity of the cotton root system in relation to changes in temperature has been fully investigated. A concept of thermal stress in plants links the biochemical characteristics of a plant with its optimal temperature range. The Thermal Kinetic Window (TKW) concept uses the temperature characteristics of an enzyme kinetic parameter, the Michaelis constant (K_m), to define the limits of thermal stress. These measures are used as indicators of metabolic efficiency. The TKW values have been identified for several crop species,[2,3,7,9] and are narrower than plant temperatures experienced on a seasonal or daily basis.[2] When plants are at temperatures outside their TKW, reduction in growth and development occurs.[2] The TKW for cotton has been reported to be between 23.5 and 32°C.[2] Growth may not be equal within the TKW range, but is significantly reduced outside the temperature limits.

The present study was conducted to investigate the temperature responses of cotton and sunflower roots, and to evaluate metabolic responses to seedling age and root/shoot thermal environments within and below the TKW. It is postulated that the temperature dependency of the metabolic activity in cotton is developmentally regulated.

MATERIALS AND METHODS

Cotton (*Gossypium hirsutum* L.) cv 'Paymaster HS26' seedlings were utilized for these studies. Seeds of each species were germinated in 17.4 cm high × 16.4 cm wide polyethylene growth pouches (Seed Pack Growth Pouches, Vaughn's Seed Co.) inside a constant temperature incubator. Two to four seeds were planted in each pouch. Each pouch contained an absorbent paper insert that was folded into a 'v' at the top. Perforations in the bottom of the 'v' allowed the roots to grow down between the paper insert and the wall of the pouch. Seeds were placed in the 'v' and aligned with tweezers to orient properly the roots upon germination. Hoagland's solution (50 μl, 1/2 strength) was added to each pouch prior to planting the seed to allow the paper insert to absorb the solution and provide a moist medium for seed imbibition, germination and seedling growth. Each pouch was suspended upright in a wooden rack that would accommodate 100–150 pouches. The racks were kept inside the incubator without light for the duration of the experiment (10 days).

Twenty random seeds of each species were planted for each temperature regime, and a subsample of 16 primary roots (four pouches containing four seedlings) were measured for primary root length, lateral root length, and number of lateral roots. The root lengths were measured by placing a ruler on the outside of the pouch along a taproot or lateral root and recording root length to the nearest millimeter. The seedlings were grown at constant temperatures ranging from 20 to 40°C in 5°C increments and evaluated after 10 days growth at each temperature. It was determined in preliminary experiments that germination and growth were significantly reduced below 20°C.

To determine the influence of shoot *versus* root temperature on the growth of the root system and the relative contribution of the root temperature on the growth of the whole seedling, studies were conducted in controlled temperature incubators, one of which was equipped with a constant temperature water bath. Two cotton seeds were planted in each of 16 pouches of each shoot/root combination for evaluation. Racks of seed were initially placed in a 28°C incubator and seedlings were allowed to develop for three days; they were then placed in incubators and allowed to grow for an additional seven days in the dark. A water bath was placed inside one of two constant temperature incubators and the pouches (16 per treatment) were suspended on racks inside the baths. The air temperature inside the incubators was maintained at either 20 or 28°C while the water bath (root temperature) was maintained at the second temperature, thus providing shoot/root temperature combinations of 28/20, 20/28, 28/28 and 20/20°C. Test temperatures of 20 and 28°C were chosen because these temperatures are common night and day temperatures for irrigated cotton on the High Plains, and because 28°C is the midpoint of cotton's TKW and 20°C is below the TKW. Seedlings exposed to either a constant 20 or 28°C temperature were placed directly in the incubators and not the water bath. Seedlings were harvested and the lengths of the taproot and lateral roots were measured at the end of the seven-day period for a total of 10 DAP. The roots were then separated from the hypocotyl and fresh weights of the roots, hypocotyl and cotyledons were measured. Since all the plants were grown for three days at 28°C before being placed in the different temperature regimes, taproot lengths were measured on 10 separate plants grown only at 28°C for three days. At the end of the three-day period, the length of the taproots was measured, and the average lengths subtracted from the final taproot lengths of the 10-day-old seedlings in order to evaluate differences in growth that occurred only after the plants had been exposed to the different temperature regimes.

Metabolic activity in the root tips was measured by the reduction in an electron acceptor 2,3,5-triphenyltetrazolium chloride (TTC).[11] In each experiment, which are described below, the attached cotton roots were placed on 3 mm filter paper (Bio-Rad #165-0962, Bio-Rad Laboratories, Richmond, CA) saturated with a solution containing 0.8% TTC in 50 mM potassium phosphate buffer, pH 7.1. The filter paper pads were located on top of temperature blocks (thermoelectric cells) of a Cellular Thermoelectric Controller (CELTEC). The eight blocks of the unit were set at 10, 15, 20, 25, 30, 35, 40 and 45°C and maintained within ± 0.5°C. Transparent plastic CO_2-permeable film was placed over the roots during the 30-min dark treatment to prevent water loss yet permit gas exchange. Following the 30 min treatment, the root tips (0.5 cm) were excised, rinsed with deionized water, and placed in 200 μl of 95% ethanol. After a 24 hr dark incubation period, the level of TTC reduction was determined by the absorbance of the ethanol extract at 490 nm.

In the first experiment, the impact of temperature on the reduction of TTC in root tissue was evaluated. Seeds of both cotton and sunflower were germinated in the growth pouches as previously described. After three days, the seedlings (eight seedlings per temperature block) were transferred to the CELTEC for evaluation of TTC reduction. Another set of seedlings of the same age was heat treated by placing the seedlings in water at 45°C for 5 min before exposure to TTC on the CELTEC temperature blocks to determine the impact of high temperature on uncoupling of electron transport in the roots.

The second experiment was designed to evaluate the effect of reduction in stored seedling reserves on the temperature response of root metabolism. Seeds of cotton were germinated in the growth pouches and allowed to grow in the dark at 28°C. Eight seedlings per temperature block were evaluated for TTC reduction at 10, 15, 20, 25, 30, 35, 40 and 45°C at three and seven DAP. The TTC reduction was expressed as a function of temperature for each age seedling and as a percentage of the temperature response of TTC reduction at three days after planting.

Experiments were arranged in a completely randomized design and analysis of variance was used to test for significance between treatments. The variability associated with each data point on the figures represents the 95% confidence limits around that point.

RESULTS AND DISCUSSION

The temperature providing maximum root length and root number in cotton seedlings 10 days after planting was 35°C (Fig. 1). Lateral roots were not apparent in cotton seedlings grown at 20°C for 10 days. Other experiments (data not shown) have indicated that if the seedlings were grown for longer time periods (15–20 days), lateral roots were initiated. There was no measurable development of cotton taproots below 20°C during the 10-day evaluation period. The development of the roots was also reduced above 35°C.

The pattern of root development in response to shoot/root temperatures of 28/20, 20/28, 28/28 and 20/20°C is shown in Table 1. Maximum root development in cotton occurred at 28/28°C with a total root length twice that of any other treatment. The treatment providing the next best root development was 20/28°C. No significant difference in root length (Duncan's Multiple range test at 0.05 probability level) was observed in the 28/20°C and 20/20°C treatments; however, roots

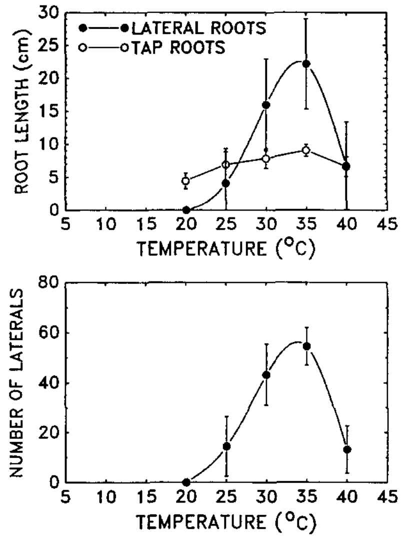

FIG. 1. Root development of cotton seedlings as influenced by temperature. Vertical bars about each mean = 95% confidence limits.

grown at the lower temperatures were thicker (fresh weight per unit length) than those grown at the higher temperatures (data not shown). Lateral root development responded dramatically to root incubation temperature. Seedlings with root temperatures of 28°C produced lateral roots, while no lateral roots developed during the test period in seedlings with root temperatures of 20°C. Total plant fresh weight was reduced when shoots and roots were grown at 20°C (0.27±0.01 g) compared with plants grown with roots and shoots at 28°C (0.64±0.01 g) (Duncan's multiple range test at 0.05 probability level).

In our studies the temperature providing maximum cotton root development during the first 10 days after planting (Fig. 1) appeared to be 3°C above the 32°C upper limit of cotton's TKW. Because of this apparent discrepancy between the reported optimal temperatures described by the TKW and the observed temperatures for maximum root growth, experiments evaluating *in vivo* enzyme temperature characteristics were initiated. Metabolic activity in root tips measured by the reduction of the electron acceptor 2,3,5-triphenyltetrazolium chloride (TTC) showed an exponential increase in TTC reduction as temperature increased (Fig. 2). In an effort to evaluate the degree of electron transport coupling associated with elevated temperatures, seedlings were evaluated after incubation in solutions containing mitochondrial uncouplers. The use of uncouplers on whole seedlings was abandoned, however, because of poor incorporation into intact roots. A second method for uncoupling electron transport in intact tissues was developed. It entailed the use of a 5 min 45°C seedling pretreatment. Uncoupling of electron transport in response to high temperature stresses has been reported numerous times.[12] The results of using a heat pretreatment to uncouple electron transport showed that a significant portion (95% confidence bars) of the roots increased ability to reduce TTC at high temperatures was associated with an uncoupling of electron transport resulting in lower metabolic efficiency. This is demonstrated by the differences in activity between control and heat treated roots (Fig. 2). The highest efficiency for electron transport in the heat treated roots measured as a percent-

Table 1. *Development of* 10-*day-old cotton seedlings grown at four temperature regimes*

Treatment Shoot temp.	Root temp.	Tap root length	Lateral root length	Total root length	No. laterals
°C		cm	cm	cm	
28	20	3.08±0.24a*	0.0a	3.08±0.24a	0.0a
20	28	5.06±0.28b	1.83±0.35b	6.89±0.54b	8.00±0.83b
28	28	7.71±0.59c	5.82±1.15c	13.53±1.07c	8.38±0.90c
20	20	4.71±0.35ab	0.0a	4.71±0.35a	0.0a

*Means followed by the same letter are not significantly different at the 0.05 probability level based on a Duncan's Multiple Range Test.

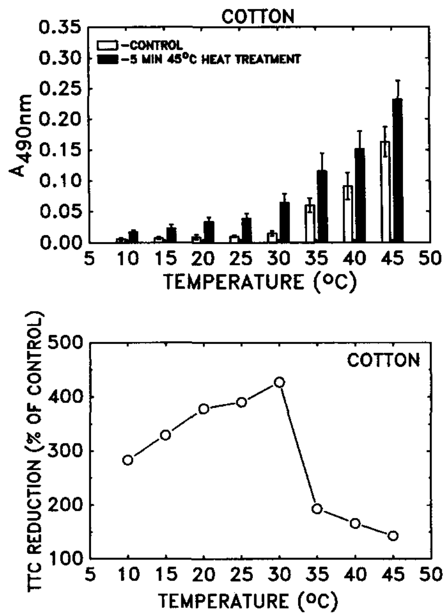

FIG. 2. The effect of heat treatment on the reduction of TTC in cotton seedlings exposed to various temperatures and expressed as a percentage of the control. Vertical bars about each data bar = 95% confidence limits.

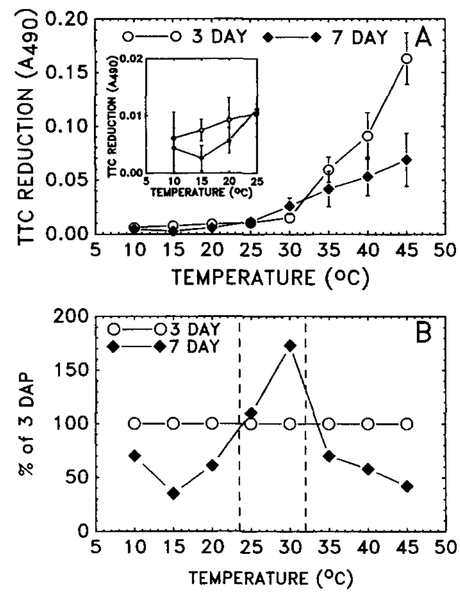

FIG. 3. The effect of seedling age on the reduction of TTC in cotton roots exposed to various temperatures. (A) Three- versus seven-day-old seedlings. Vertical bars about each mean = 95% confidence limits; (B) TTC reduction in seven-day-old seedlings expressed as a percentage of the three-day-old seedlings.

age of the control activity occurred at 30°C.

A comparison of the metabolic temperature characteristics of intact roots of three- and seven-day-old cotton seedlings monitored by the *in vivo* reduction of TTC revealed different temperature response curves (Fig. 3A). The rate of TTC reduction in root tips at seven days was more temperature sensitive (Fig. 3A insert) than the activity in root tips of seedlings at three days. Similar activities were observed in the 10, 25 and 30°C treatments. These results are consistent with the observation that optimum enzyme efficiency occurs within the TKW previously established for cotton (Fig. 3B vertical lines).[2] This suggests that there is a developmental regulation of the temperature response. This could be related to the changes in mobilization of seed reserves that occur as seedlings develop.[4] BURKE and OLIVER[4] have shown in cucumbers, for example, that suboptimal temperatures acted to delay germination, change cotyledonary soluble sugars and broadened the temperature range for chlorophyll accumulation. The broadening of the temperature characteristics of TTC reduction during the period of rapid mobilization of stored reserves may help to provide an explanation for the observed shift from the predicted TKW of 23.5 to 32°C to the maximum root production at 35°C after 10 days. The data in Fig. 1 show an optimal temperature of 35°C for root growth in cotton; however, as BURKE and OLIVER[4] have pointed out, the temperature response of development may be strongly dependent upon reserves stored in the cotyledons. Thus the optimum temperature that we report here must be tempered by the caveat that it is a composite value derived over the first 10 days of growth when there is a strong influence of stored reserves. We suggest that the data presented in Fig. 3 support this postulate and indicate that the actual temperature optima for root growth in cotton is lower than 35°C. Clearly, the temperature sensitivity of early root growth will be slightly higher than the metabolic optimum for enzyme function during most of the growing season because of the availability of the rapidly mobilized seed reserves.

In summary, we have shown differences in temperature characteristics of root growth responses in young cotton seedlings. Root metabolism may become more temperature sensitive as the mobilization of cotyledonary reserves declines during early seedling growth indicating that the temperature dependency of the metabolic activity is developmentally regulated. These data also suggest that root growth in response to temperature is maximal within the optimal temperatures predicted from the TKW. These findings suggest the need for a re-evaluation of the use of the temperature characteristics of seedling root development to identify optimal temperatures for growth and development.

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